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Synthesis and Calcium-Antagonist Activity of some phosphonates

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The synthesis and pharmacological evaluation of a series of phosphonates related to Fostedil, diethyl 4-(benzothiazol-2-yl)benzylphosphonate **1**, a potent calcium antogonist are reported. Among the compounds studied, only diethyl 4-(benzooxazol-2-yl)benzylphosphonate **5**, which is closely related to Fostedil, shows a low calcium-antagonist activity.

Synthese und Calcium-antagonistische Wirkung einiger Phosphonate

Synthese und pharmakologische Bewertung von Phosphonaten, die mit dem Calcium-Antagonisten Phostedyl, Diethyl-4-(benzothiazol-2-yl)-benzylphosphonat, verwandt sind, werden beschrieben.
Unter den untersuchten Verbindungen zeigt nur Diethyl-4-(benzoxazol-2-yl)-benzylphosphonat **5** das O-Analoge des Phostedyls eine niedrige Ca-antagonistische Aktivität.

Calcium-antagonists are compounds that interfere with calcium flux across cellular membranes[1]. Verapamil[2], Nifedipine[3] and Diltiazem[4] are calcium-antagonists used to treat atherosclerosis, hypertension and myocardial infarction. During researches on derivatives of dialkyl phosphonates, which show vasodilating effect[5], *Kohno* and coworkers[6] have synthesized the phosphonate **1**, named Fostedil, which shows calcium-antagonist activity comparable to that of Diltiazem.

Our object has been the synthesis of some new phosphonates in order to check if the modification of the structure of Fostedil could preserve the calcium-antagonist activity.

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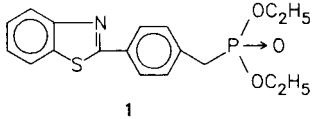
First of all we have synthesized the isoster **5** of Fostedil by condensing 2-aminophenol **2** with 4-(bromomethyl)benzoic acid **3** in PPE (polyphosphate ester)[7] and by transformation of **3** into 4-(benzoxazol-2-yl)bromomethyl-benzene **4** with triethyl phosphite by *Michaelis-Arbuzov* reaction.



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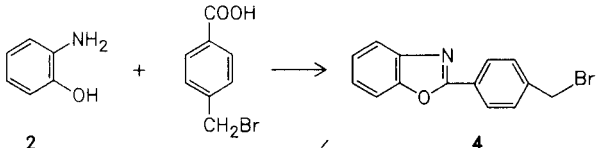
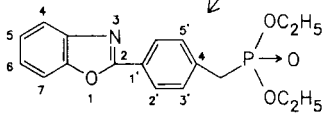


2 **3** **4**



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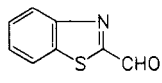
Subsequently we have synthesized compounds **8** and **9** where the phenyl ring has been eliminated.

7 was synthesized by *Wittig* reaction between 2-thiazolecarboxaldehyde **6**[8] and diphenyl triphenylphosphoranylidenemethyl phosphonate in benzene according to Jones[9]. **7** was converted into the diethyl ester **8** with NaOEt in anhydrous EtOH. **9** was synthesized by reaction of 2-thiazolecarboxaldehyde **6** with diethyl phosphite and triethylamine.



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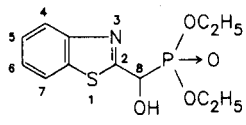

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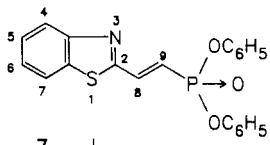

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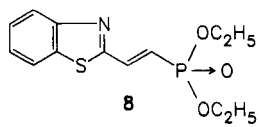

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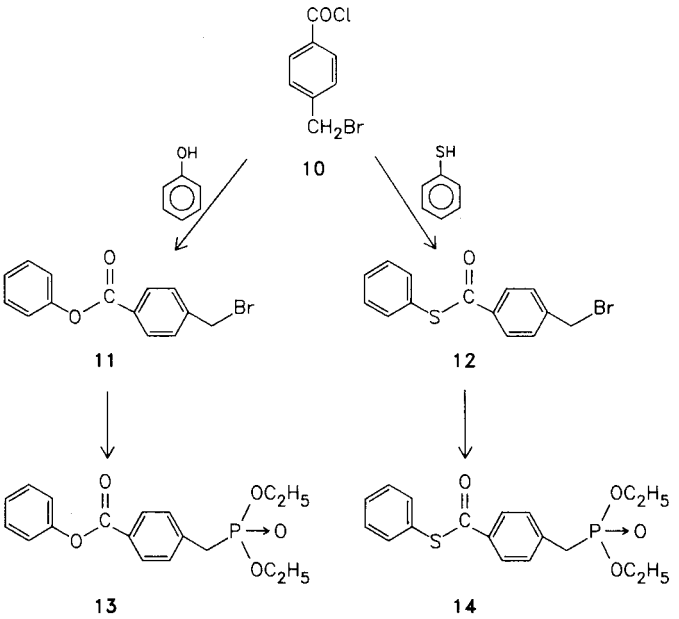
Finally we have synthesized compounds **13** and **14** where we have linked to the benzylphosphonate group lipophilic groups like ester and thioester. These compounds were prepared starting from α-bromo-p-toluic acid which was transformed into the chloride **10** which in turn afforded, by reaction with phenol and thiophenol, esters **11** and **12**. These esters, by *Arbuzov* reaction with triethyl phosphite, were transformed into the phosphonates **13** and **14**.



Pharmacology

Materials and Methods

Strips of tenia coli (2–2.5 cms) drawn from adult guinea pigs, weight 250–300 g (Rodentia allevamenti; Brescia), have been suspended in baths for isolated organs, 10 ml containing depolarizing Tyrode, not containing $CaCl_2$ composed as follows: (mmol/L): NaCl 97, KCl 40, $NaHCO_3$ 11.9, $NaH_2PO_4 2H_2O$ 0.4, glucose 5.5, pH 7.1 at 37° areated with O_2 95 %–CO_2 5 %. – A basal tension of 1 g has been applied to the above described strips and this has been kept for the whole duration of the experiments. – The inhibition of the contracting effect of the sub-maximal concentration of $CaCl_2$ (3 mM) in presence of each concentration of the tested compounds has been estimated. – Where possible, the IC_{50} (inhibiting concentration of 50 % of the sub maximal effect) has been estimated, using the method of the linear regression.

Tab.: Calcium-antagonist activity determined on taenia coli of guinea pigs.

Compound	conc. μM	experiment N°	% inhib.[a] M ± S.D.	IC_{50}[b] μM
Nifedipine	$0.3 \cdot 10^{-3}$	5	20.3 ± 5.3	$1.04 \cdot 10^{-3}$
	$1.0 \cdot 10^{-3}$	5	47.1 ± 7.1	
	$3.0 \cdot 10^{-3}$	5	77.1 ± 5.6	
13	0.1	3	0	> 1
	1.0	3	18.6 ± 20.1	
14	0.1	3	0	> 1
	1.0	3	8.8 ± 14.1	
8	0.1	3	10.6 ± 2.9	> 1
	1.0	3	19.6 ± 5.3	
9	0.1	3	3.6 ± 3.1	> 1
	1.0	3	15.0 ± 6.2	
5	0.1	3	17.9 ± 2.7	3.8
	1.0	3	73.2 ± 6.4	

a) Average values with the pertinent standard deviations (M ± S.D.) of the % inhibitions of the sub-maxim. contractions induced by $CaCl_2$ obtained for each concentration of the tested compound.
b) Inhibiting concentration of 50 % of the sub-maxim. effect, using the method of linear regression.

The pharmacological results reported in the table show that only the isoster **5** of Fostedil preserves a low biological activity as calcium-antagonist.

Compounds **7** and **9** where the phenyl ring has been eliminated and compounds **13** and **14** where the benzothiazolic group has been substituted with liphophilic groups do not show any activity.

Acknowledgement

We are grateful to Glaxo S.p.A. for the pharmacological determination; this work was supported by the Ministry of Public Education.

Experimental Part

MP: Reichert microhotstage; uncorr. – IR: Beckman Acculab 5 IR spectrophotometer (CCl_4). – ^{1}H-NMR spectra: Varian EM 390, 90 MHz spectrometer, $CDCl_3$ as solvent, TMS int. stand. – MS: Varian Mat 311; all compounds show parent peaks identical to theoretical values. – Purity was checked by TLC.

2-4-(Bromomethyl)phenylbenzoxazole **(4)**

To a heated mixture of 1.09 g (0.01 mole) of 2-aminophenol and PPE (10 g) ($3 \cdot 10^{-2}$ mole) at 100°, was added α-bromo-p-toluic acid, 3.2 g ($1.5 \cdot 10^{-2}$ mole). After heating at 100° for 1 hour the reaction mixture was cooled, diluted with ice water, and neutralized with $NaHCO_3$. The precipitate was purified on silica gel, using 1:1 hexane/ether as eluent, to give compound **4**, yield 50 %; mp. 160–165°. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.5 (s, 2H; CH_2Br), 7.0–7.5 (m, 6H aromat., H-2',3',5',6',5,6), 8.0–8.2 (m, 2H aromat., H-4,7). – $C_{18}H_{10}BrNO$ (288) Calc. C 58.3 H 3.4 N 4.8 Found 58.1 H 3.1 N 4.5.

Diethyl 4-(benzoxazol-2-yl)benzylphosphonate **(5)**

A mixture of 0.3 g ($1 \cdot 10^{-3}$ mole) of **4** and 0.6 ml of triethyl phosphite was heated at 130° for 30 min. After cooling to room temp., the resulting solid was chromatographed on silica gel using as eluent hexane, or hexane/CH_2Cl_2 9:1, respectively, to give **5**, yield 29 %; mp 65–70°. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.2 (t, 6H; J=7.2 Hz, 2 P-$OCH_2\underline{CH_3}$), 3.2 (d, 2H; CH_2-P-), 4.1 (quint., 4H; J = 7.2 Hz, 2 $PO\underline{CH_2}CH_3$), 7.0–7.5 (m, 6H aromat., H-2′,5′,3′,6′,5,6), 8.0–8.1 (m, 2H aromat., H-4,7). – $C_{18}H_{20}NO_4P$ (345) Calc. C 62.6 H 5.7 N 4.0 Found C 62.9 H 6.0 N 4.4.

Diphenyl β-(benzothiazolyl-2-)vinylphosphonate **(7)**

A mixture of 1.6 g diphenyl triphenylphosphoranylidenemethylphosphonate ($3 \cdot 10^{-3}$ mole) and 0.5 g ($7 \cdot 10^{-3}$ mole) 2-thiazolecarbaldehyde **6** in anhydrous benzene (30 ml) was heated at 80–90° for 7 h. The solvent was evaporated in vacuo and the crude residue chromatographed on silica gel using Et_2O as eluent. The solid obtained was crystallized from EtOH, yield 62 %, mp 120–121°. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.3 (dd, 1H; $J_{H\text{-}H}$ = 17.0 Hz, $J_{P\text{-}H\,gem}$ = 20.2 Hz, H-9), 6.8 (d, 1H; H-8), 7.0–8.0 (m, 14H aromat., H-4,5,6,7, 2 P-OC_6H_5). – $C_{21}H_{16}NO_3S$ (362) Calc. C 69.6 H 4.4 N 3.8 Found C 69.3 H 4.1 N 3.4.

Diethyl β-(benzothiazolyl-2-)-vinylphoshonate **(8)**

To a stirred solution of 0.9 g ($2 \cdot 10^{-3}$ mole) of **8** in anhydrous EtOH (10 ml), was added in a few min a solution of NaOEt prepared from 0.16 g ($7 \cdot 10^{-3}$ mole) of Na in 5 ml of anhydrous EtOH. The mixture was stirred at room temp. for 5 h and then poured into water saturated with NaCl, and extracted several times with CH_2Cl_2. The extracts were washed with water and dried on Na_2SO_4. CH_2Cl_2 was removed in vacuo and the crude product was chromatographed on SiO_2, using 3:1 benzene-Et_2O as eluent to give compound **8**; yield 50 %. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.5 (t, 6H; J = 7.2 Hz, 2 P-$OCH_2\underline{CH_3}$), 4.0–4.3 (quint., 4H; J = 7.2 Hz, 2 P-$O\underline{CH_2}CH_3$), 6.8–7.0 (pseudo-t, 1H; CH=$\underline{CH}$-P, $J_{H\,H}$ = $J_{P\text{-}Hgem}$ = 17.3 Hz), 7.5–8.0 (m, 5H, $\underline{CH}$=CH, 4H aromat.). – $C_{13}H_{16}NO_3SP$ (297) Calc. C 52.5 H 5.3 N 4.7 Found C 52.8 H 5.6 N 5.0.

Diethyl (hydroxy, benzothiazol-2-yl)methylphosphonate **(9)**

The mixture of 0.34 g ($2 \cdot 10^{-3}$ mole) of 2-thiazolecarboxaldehyde **6**, 0.270 g ($2 \cdot 10^{-3}$ mole) of diethyl phosphite, and 0.1 g ($1 \cdot 10^{-3}$ mole) of Et_3N in 5 ml of Et_2O was stirred at room temp. for 24 h; after filtration, the solvent was removed in vacuo. The solid residue was crystallized from Et_2O giving a white product, yield 17 %, mp 134–35°. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.3 (t, 6H; J = 7.3 Hz, 2 P-$OCH_2\underline{CH_3}$), 4.2 (quint., 4H; J = 7.3 Hz, 2 P-$O\underline{CH_2}CH_3$), 5.6 (d, 1H, CHOH), 6.3 (s, 1H; OH), 7.3–8.0 (m, 4H; aromat.). – $C_{12}H_{16}NO_4SP$ (301) Calc. C 47.8 H 5.3 N 4.6 Found C 47.3 H 5.0 N 4.2.

Phenylester of 4-bromomethyl benzoic acid **(11)**

A mixture of 1 g ($4 \cdot 10^{-3}$ mole) α-bromo-p-toluic acid and 1 g ($8 \cdot 10^{-3}$ mole) of $SOCl_2$ was heated at 70° for 12 h. Excess of $SOCl_2$ was removed in vacuo and the chloride was stirred with 0.42 g ($4 \cdot 10^{-3}$ mole) of phenol at 80–90° for 3 h. The product was extracted with $CHCl_3$, and the org. layer was washed with 1N NaOH, water, and dried over Na_2SO_4. Evaporation of the solvent gave a white solid which was crystallized from Et_2O, yield 43 %, mp 105–110°. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.35 (s, 2H; CH_2Br), 7.0–7.4 (m, 7H aromat., H-2,3,5,6,3′,5′,4′), 8.0 (m, 2H aromat., H2′,6′). – $C_{14}H_{11}BrO_2$ (291) calc. C 57.7 H 3.7 Found C 57.4 H 3.6.

Thiophenyl ester of 4-bromomethyl benzoic acid **(12)**

2.15 g ($1 \cdot 10^{-2}$ mole) of α-bromo-p-toluic acid and 4.72 g ($4 \cdot 10^{-2}$ mole) of $SOCl_2$ were heated at 70° for 12 h. Excess of $SOCl_2$ was removed in vacuo and to the residue was added 1.1 g (0.01 mole) of thiophenol; the mixture was stirred at 80° for 3 h. The product, after cooling, was extracted with $CHCl_3$, and the org. phase was washed with 1 N NaOH, water and dried over Na_2SO_4. After evaporation of the solvent in vacuo a solid residue was obtained in crystalline form after crystallization from hexane-Et_2O, yield 32 %, mp 100–105°. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 4.4 (s, 2H; CH_2Br), 7.3 (m, 7H aromat., H-2,3,5,6,3′,4′,5′), 7.8 (m, 2H aromat., H-2′,6′). – $C_{14}H_{11}BrOS$ (307) Calc. C 54.7 H 3.5 Found C 54.5 H 3.7.

Diethyl4-(carbophenoxy)benzylphosphonate **(13)**

0.5 g ($2 \cdot 10^{-3}$ mole) **11** and 0.83 g ($5 \cdot 10^{-3}$ mole) of triethyl phosphite were heated at 80° for 3 h. The product was chromatographed on silica gel, using Et_2O, Et_2O/CH_2Cl_2 9:1 as eluent, giving a white ctystalline product, yield 43 %, mp 95–100°. – ^{1}H-NMR =$CDCl_3$): δ (ppm) = 1.3 (t, 6H; J = 7.2 Hz, 2 P-$OCH_2\underline{CH_3}$), 3.2 (d, 2H; CH_2-P-), 3.8 (quint., 4H; J = 7.2 Hz, 2 P-$O\underline{CH_2}CH_3$), 7.0–7.5 (m, 7H aromat., H-2,3,6,5,3′,4′,5′), 8.0 (m, 2H aromat., H-2′,6′). – $C_{18}H_{21}O_5P$ (348) Calc. C 62.0 H 6.0 Found C 62.4 H 6.3.

Diethyl 4-(Phenylthiocarbonyl)benzylphosphonate **(14)**

A mixture of 0.6 g ($2 \cdot 10^{-3}$mole) of thioester **12** and 0.6 g ($3 \cdot 10^{-3}$ mole) of triethyl phosphite was heated at 100° for 3 h. The mixture was allowed to cool to room temp. and the resulting oil was chromatographed on silica gel column, using $Et_2O/CHCl_3$ 1:1 as eluent, a white product was obtained; yield 55 %, mp 90–95°. – ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.3 (t, 6H; J=7.2 Hz, 2 P-$OCH_2\underline{CH_3}$), 3.2 (d, 2H; CH_2P-), 4.0 (quint., 4H; J=7.2 Hz, 2 P-$O\underline{CH_2}CH_3$), 7.3 (m, 7H aromat., H-2,3,4′,5,6,3′,5′), 7.8 (m, 2H aromat., H2′,6′). – $C_{18}H_{21}O_4PS$ (364) Calc. C 59.3 H 5.7 Found C 59.0 H 5.9.

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